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Species selective interaction of Alphaherpesvirinae with the "unspecific" immune system of the host

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Summary. During evolution Herpesviridae have developed glycoproteins, which interact with essential components of the immune system. Besides immunoglobulin-binding proteins (= Fc-receptors), expressed by several members of the herpesfamily, the interaction with the complement system plays a role in the pathogenicity of herpes simplex virus. Here we report that the ability to interact with the third complement component (C3), the central mediator of complement activation, was also found among several animal alphaherpesviruses. This interaction appeared to be species-selective as the viral proteins preferentially bound to the C3 originated from the respective host. That could provide a possible explanation for the evolution of a variety of herpesviruses as the species tropism observed among Herpesviridae may be influenced by specific adaptation of protective virus-proteins to the immune system of the different hosts. The data have critical implications for the studies of virus host interactions in heterologous systems and support a role for the C3-binding proteins in pathogenesis. Since the C3-binding proteins are conserved among different herpesviruses they could serve as suitable subunit-vaccine candidates.

Introduction

The complement system is important in the host response to foreign pathogens [24]. Originally thought to be a rather "unspecific" defense mechanism completing the antibody function, it even provides some self and nonself discrimination function due to the specific characteristics of complement-regulatory proteins on the cell surface and in the serum [2]. Herpesviruses have been shown to express immunoglobulin-binding proteins (= Fc-receptors) at the surface of infected cells [25] but in the early phases of infection, complement activation proceeds mainly by the antibody-independent alternative pathway. Cells in-

ected with herpes simplex virus (HSV), bovine herpesvirus (BHV), and a variety of other viruses activate this pathway [5, 13, 38]. However, in vitro HSV-infected cells are rather resistant to complement lysis in the absence of antibody [15, 38]. This seems to be due to the function of HSV glycoprotein gC which interacts with C3, the pivotal component of the complement cascade [7, 9, 28], and thereby inhibits alternative pathway complement activation [11, 15, 17].

Glycoproteins gC of both HSV serotypes share amino acid homology with g III of pseudorabiesvirus (PRV) and g III of bovine herpesvirus type 1 (BHV-1), gpV of varicella-zoster virus, A-antigen of Marek's disease virus, and gp 13 of equine herpesvirus type 1 (EHV-1) [8]. Although the g IIIs and their homologues are not essential for viral replication in vitro, they appear to be of major importance in vivo [3, 10, 12, 35]. Glycoproteins g III of PRV and BHV-1 have been reported to mediate virus attachment to heparin-like domains on the target cell surface [29, 32] as well as HSV gC [16, 32]. Heparin blocks the binding of virion associated gC to these cell-"receptors" [16] and, of interest, heparin also inhibits interactions between C3 and gC [22].

In previous studies we have tested the binding of sheep erythrocytes coated with C3 of different species (EAC) to HSV-1 infected cells and found a marked preference for the human complement component [20]. In addition, we could show that PRV g III binds to swine complement component C3, but only showed weak binding to the human complement factor [21]. Therefore we asked if the g III homologues from other herpesviruses also show species specificity for the interaction with C3.

Material and methods

FACS analysis

Vero cells were infected with HSV-1 strain WAL [33] at a multiplicity of infection of > 5 . 24 h later, anti-gC monoclonal antibody (moAb) HC 1 [19] was added at a 1:100 dilution followed by fluorescein labeled F(ab)₂ goat anti-mouse serum (Cappel Lab., Cochranville, PA). The porcine kidney cell-line PK 15 was infected with PRV strain Becker [30] for 24 h and expression of g III determined using anti-g III moAb M 1 [14]. Bovine kidney cell line MDBK was infected with BHV-1 strain LA (American Type Culture Collection, Rockville, MD) for 24 h and pooled anti-g III moAbs were used to detect g III. Controls consisted of a moAb directed against an irrelevant antigen (anti-CD4; Ortho Diagnostic Systems, Raritan, NJ).

Purification of virusproteins

Production of purified BHV-1 g III has been described elsewhere [37]. Purified HSV-1 gC was kindly donated by Drs. G. Cohen and R. Eisenberg. PRV g III was prepared by immune-affinity chromatography according to the method used for purification of gC [7]. Briefly, PK-15 cells were infected with PRV strain Becker, cell extracts prepared, and g III affinity purified using moAb M 1 [30] coupled to CNBr-Sepharose (Pharmacia, Uppsala, Sweden). The elution yielded a predominant 60 kDa and lesser amounts of 74 kDa and 92 kDa proteins.

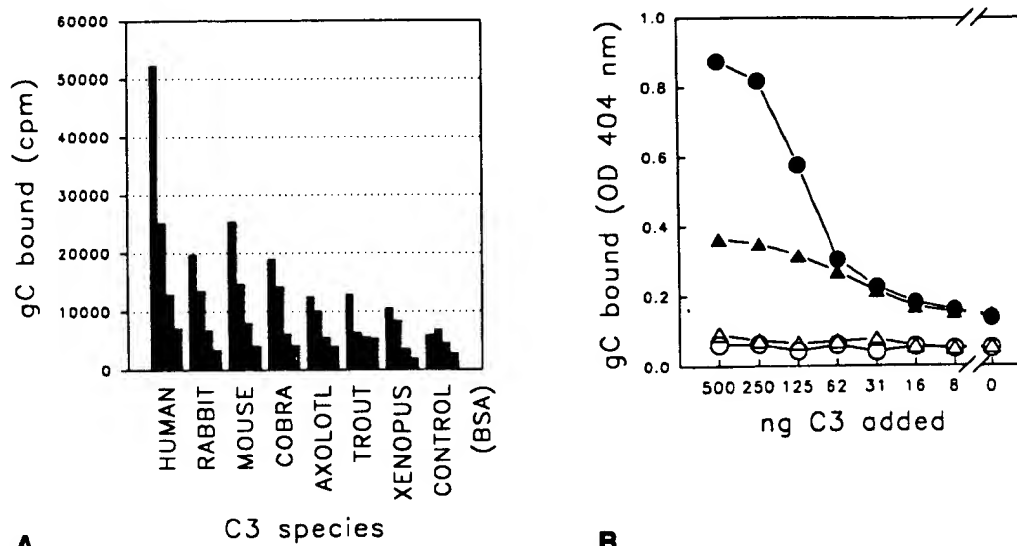


Fig. 1. Binding of HSV-1 glycoprotein C to the complement component C3 from different species. **A** Equal amounts of purified C3 from different species were coupled to RIA-plates and binding of two-fold dilutions of labeled gC was tested. **B** Guinea pig C3 was obtained subsequently and binding of gC to different amounts of human (● with, ○ without gC) and guinea pig C3 (▲ with, △ without gC) was compared by ELISA. Plates coated with bovine serum albumin (BSA) served as negative control

Complement components

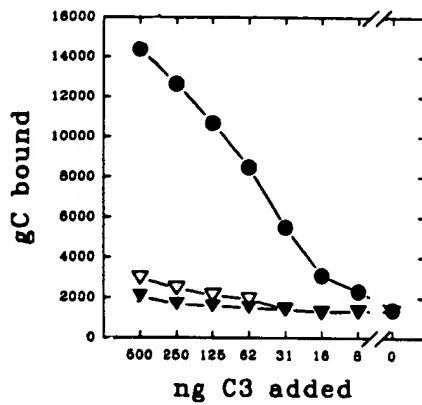
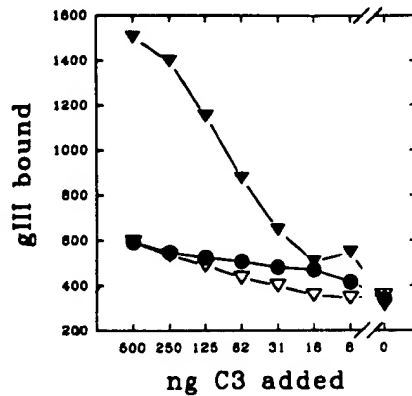
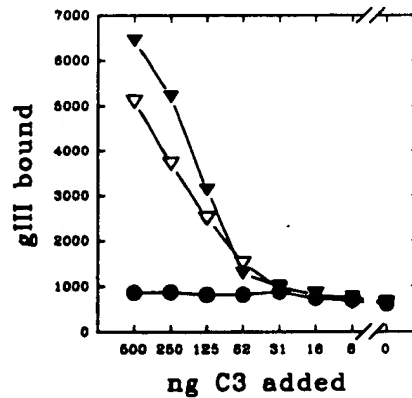
C3 from human, cow, pig, rabbit, mouse, cobra, xenopus, axolotl, or trout was purified as described [1]. Purified guinea pig C3 was a kind gift of Professor D. Bitter-Suermann (Hannover, Federal Republic of Germany). Guinea pig C3 used for the rosetting assays was obtained from Diamedix (Miami, FL).

Rosetting assays

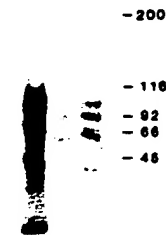
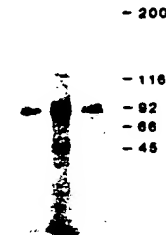
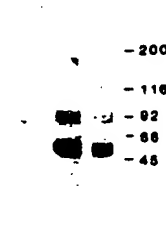
Rosetting studies with infected cells and complement coated red blood cells were performed as described previously [9, 19]. Briefly, sheep erythrocytes (E) were sensitized with sub-agglutinating concentrations of rabbit anti-sheep erythrocyte IgM. Antibody coated erythrocytes (EA) were then incubated in a stepwise manner with human complement components C1, C4, C2, and finally C3 derived from the different species. Erythrocytes lacking complement component C3 (EAC142) served as controls. Deposition of C3 on the EAC was verified using a goat anti-human C3 serum that also reacts with the other species C3 (from Cooper Biomed., Malvern, PA), and that agglutinated each of the C3-coated erythrocyte preparations (EAC). PK 15 cells were infected with PRV strain Becker and MDBK cells with BHV-1 strain LA. Vero cells were infected with HSV-1 strain WAL, EHV-1 strain Kentucky D, and the guinea pig herpesvirus (gphV) strain LK 40 [18] (both provided by the ATCC, Rockville, MD), respectively. After the infected cells had undergone complete cytopathic effect, they were removed by shaking and rosetting was performed in suspension as described [19].

Radio immuno assays

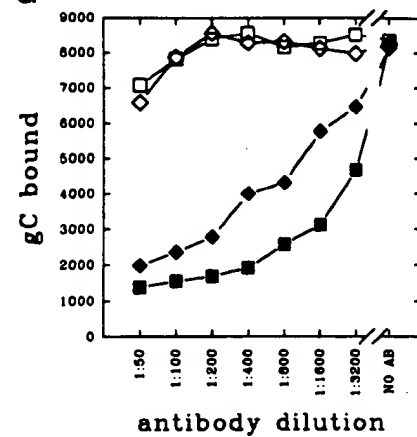
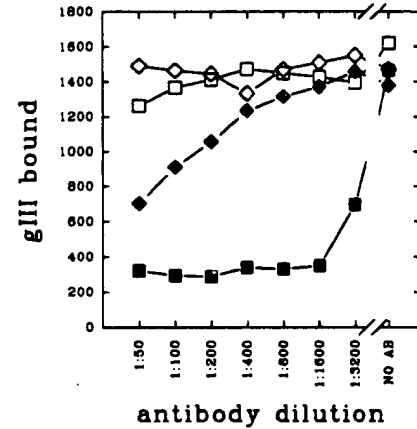
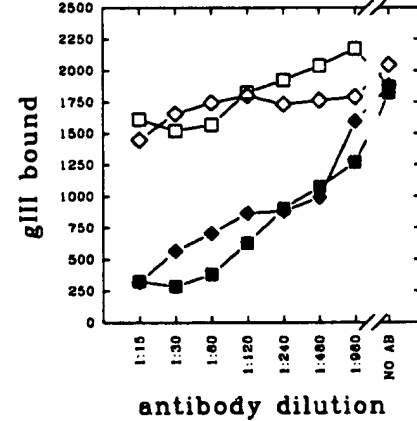
Purified glycoproteins were labeled with ^{125}I by the Iodogen method (Iodobeads; Pierce Chemicals, Rockford, IL) to a specific activity of $1-5 \times 10^6$ cpm/ μg . RIA plates (Maxisorb;

HSV-1**BHV-1****PRV**

● human C3
 ▼ porcine C3
 ▲ bovine C3

D.**E.****F.**

MW KD
 porcine C3
 bovine C3
 human C3

G.**H.****I.**

■ mAb anti gC/gIII
 □ control mAb
 ◆ anti gC/gIII serum
 ◇ control serum

NUNC, Roskilde, Denmark) were coated with 500 μ g of C3 per well for 2 h, washed twice with phosphate buffered saline (PBS) pH 7.2 containing 0.05% Tween 20 (PBST) and nonspecific binding sites were saturated with PBST supplemented with 1% bovine serum albumin (PBSTB). Subsequently labeled gC (Fig. 1 A), diluted two-fold in PBSTB, were added for 30 min at 37°C. Plates were then washed four times with PBST and bound gC determined in a γ -counter (LKB). Binding of labeled HSV-1 gC, BHV-1 gIII, and PRV gIII (200,000 cpm/well) to varying dilutions of purified human, bovine and porcine C3 fixed to RIA plates was tested in Fig. 2 A–C.

Bound glycoproteins were also analysed by SDS-PAGE. The bound radioactivity was removed from the wells by adding SDS-PAGE sample buffer (2% SDS) and the eluted material was chromatographed under reducing conditions on a 5–15% polyacrylamide gel (BioRad) (Fig. 2 D–F).

Specificity of binding was verified by blocking reactions using anti-gC moAb 1C8 [34] and a polyclonal anti-gC rabbit serum; PRV anti-gIII moAb M7 [14] and a polyclonal goat anti-gIII serum (kindly provided by L. W. Enquist); and BHV-1 anti-gIII pooled moAbs [37] and rabbit polyclonal anti-gIII serum. Labeled glycoproteins were preincubated with serial dilutions of anti-gC/gIII moAbs or polyclonal antisera and binding to the homologous C3 species (200 ng/well) was assayed as above. Controls consisted of preimmune sera and irrelevant moAbs directed against other viral glycoproteins (Fig. 2 G–I).

Enzyme immuno assay

Varying amounts of human and guinea pig C3 were added to the ELISA-plate (NUNC-Covalink), nonspecific binding sites were saturated with 100 mM Tris, 1% BSA, pH 7.4, and 100 ng of purified gC added in PBSTB for 30 min at 37°C. Plates were washed with PBST and reacted for 30 min with 1 μ g/ml anti-gC moAb HC1 [19] in saturation buffer. After washing, a 1:1000 dilution of peroxidase labeled goat anti-mouse serum (Boehringer, Mannheim, Federal Republic of Germany) was added, bound peroxidase reactivity determined by adding ABTS (azinoethyl-benzthiazolin-sulfate) and H_2O_2 , and absorbance was measured at 404 nm. To determine background values, the reaction was performed as described except no gC was added.

Results

Binding of HSV-1 gC to different species C3

Studying the binding of purified HSV-1 glycoprotein C (gC) to purified C3 of lower mammals and amphibia revealed predominant binding to human C3 if compared to C3 of different species. The binding of gC to human C3 usually

Fig. 2. Binding of gIII from BHV-1 and PRV and HSV-1 gC to human, bovine and porcine C3. A–C Purified glycoproteins gIII from BHV-1 and PRV, and gC from HSV-1, were iodinated and binding was tested to different amounts of human (●), bovine (▼) and porcine C3 (▽) coupled to RIA-plates. D–F Bound radioactivity was removed from the wells by adding SDS-PAGE sample buffer and analyzed by SDS-PAGE. Molecular weight standards are indicated. G–I Blocking of specific glycoprotein binding by anti-gIII/gC moAbs and polyclonal antisera is shown. Constant amounts of labeled glycoproteins were preincubated with dilutions of anti-gIII/gC moAbs (■) or polyclonal antisera (◆). Irrelevant moAbs (□) and preimmune sera (◇) served as controls

was three to ten-fold higher compared to the other species C3 tested (Fig. 1 A and B).

Binding of purified glycoproteins g III from PRV and BHV-1 to different C3

We purified the g III from PRV and BHV-1 and compared their ability to bind to C3 of humans, pigs, and cows (Fig. 2). Purified HSV gC was used for comparison and preferentially bound to human C3 compared with pig and cow C3 (Fig. 2 A). Similarly, purified BHV-1 g III preferentially bound bovine C3 compared with human and porcine C3 (Fig. 2 B), while PRV g III bound to pig and cow C3, but not to human C3 under the conditions tested (Fig. 2 C). The species specificity of the interactions between the g III-homologous proteins and C3 was also demonstrated by eluting the bound radiolabeled glycoproteins from wells in the RIA-plate and examining the eluate by SDS-PAGE. Considerably more gC was eluted from human C3 than from bovine or porcine C3 (Fig. 2 D). Similarly, more BHV-1 g III was eluted from bovine C3 than from heterologous C3 (Fig. 2 E). The differences between binding of PRV g III to bovine and porcine C3 were not significant but there was only background binding to human C3 (Fig. 2 F).

The specificity of the interactions between purified glycoproteins and C3 was shown by inhibition assays. Monoclonal and polyclonal anti-gC antibodies inhibited gC binding to human C3, while a control moAb directed at gD-1 or preimmune serum failed to block the interaction (Fig. 2 G). Similarly, binding of BHV-1 g III to bovine C3 and PRV g III to pig C3 was blocked by specific anti-g III antibodies (Fig. 2 H and I).

Expression of the C3-binding proteins on the surface of infected cells, rosetting assays

We examined whether BHV-1 g III or PRV g III bound C3 at the cell surface. We used flow cytometry to demonstrate that gC, PRV g III, and BHV-1 g III are expressed at the cell surface (Fig. 3 A-C). Binding of complement coated red blood cells to infected cells was tested by rosetting. EAC rosettes formed around HSV-1 infected cells (Fig. 3 D); however, none formed around PRV or BHV-1 infected cells, even though the EAC complexes contained C3 from the homologous species (Fig. 3 E and F). The same rosetting approach with two other herpesviruses, EHV-1 and the guinea pig herpesvirus (gpHV) and EAC coated with different species C3 also revealed a marked preference for the homologous C3 (Fig. 4). Thus gpHV infected cells only bound to the EAC coated with guinea pig C3 (EAC-gpig) whereas EHV-1 did not bind to EAC-gpig, but bound to the horse complement component. There was also binding of EHV-1 to the human C3.

As expected HSV-1 infected cells bound best to EAC coated with human C3 but also showed some reaction with the EAC-gpig.

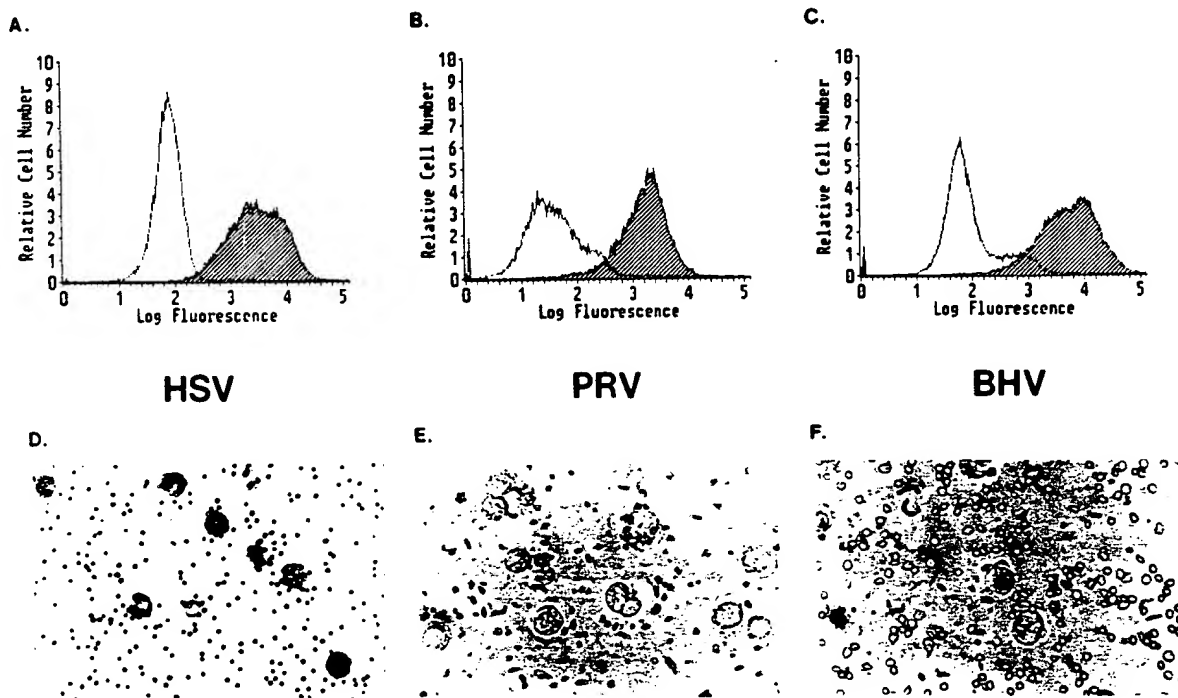


Fig. 3. Detection of the C3-binding proteins at the infected cell surface, and rosetting of infected cells with erythrocytes coated with C3 from the homologous species. Expression of HSV-1 gC (A), PRV gIII (B), and BHV-1 gIII (C) at the cell surface was tested by FACS analysis using specific moAbs (hatched curves). Irrelevant moAbs are shown for comparison (open curves). Rosetting of EAC coated with human C3 (D), porcine C3 (E), and bovine C3 (F) with HSV-1, PRV, and BHV-1 infected cells is shown below

Discussion

A major observation were the differences of HSV-1 gC binding to C3 of different species. The most probable explanation for this phenomenon is that gC has, comparable to human complement receptors, multiple binding sites on the C3 molecule [20] which are conserved only in part in the different species ([1]; Huemer et al., in press). There was also binding to a certain extent to C3 of other species if measured by rosetting assays and binding of HSV-1 and EHV-1 infected cells has been also observed to red blood cells coated with mouse complement [4]. We do not think that this contradicts our hypothesis, as the rosetting method is able to detect very low affinity interactions and there is no washing with detergent as in the RIAs.

Surprisingly PRV and BHV-1 did not lead to rosetting if tested by EAC. This is similar to the situation found with gC of HSV type 2. Although purified gC from HSV-1 and HSV-2 each bind to C3 [7, 28], only HSV-1 infected cells form rosettes with EAC [9]. Therefore it appears that the ability to form rosettes with C3 coated red blood cells is not the rule among herpes viruses [4, 34].

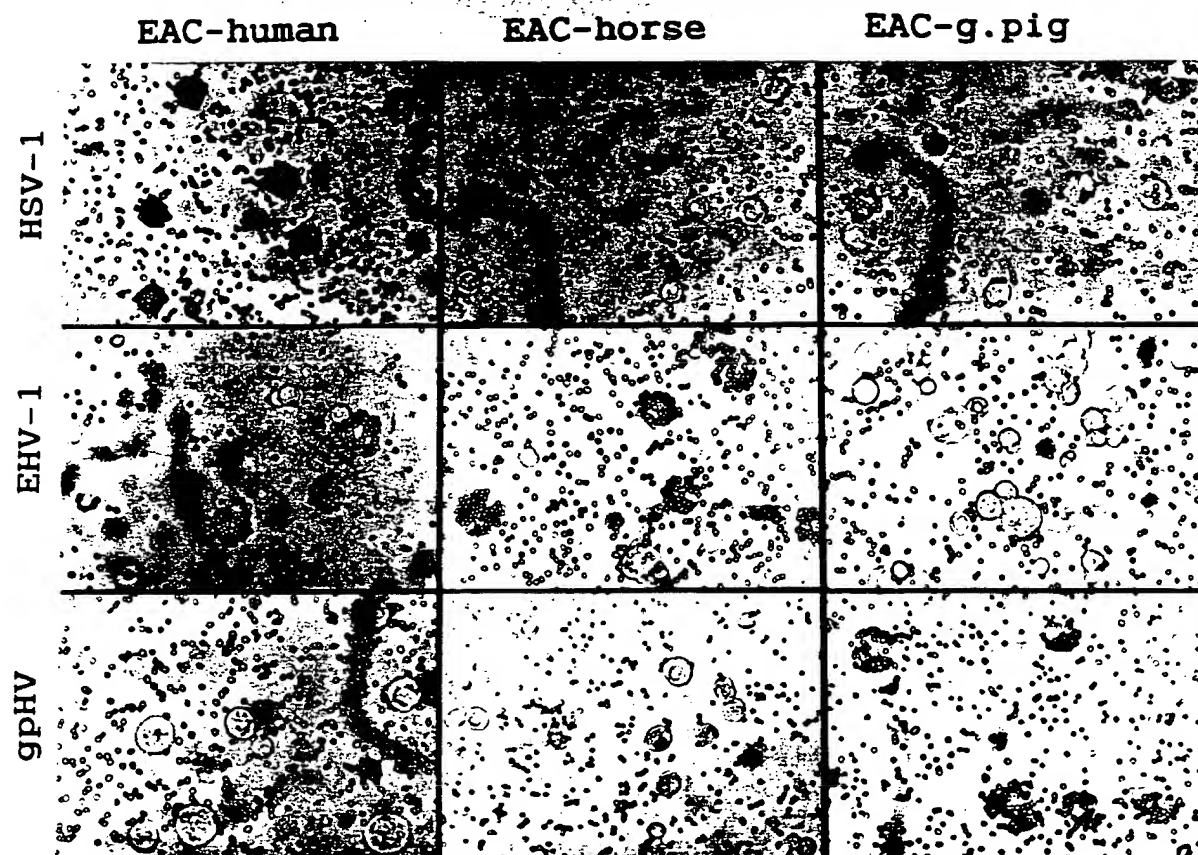


Fig. 4. Rosetting of human, horse and guinea pig C3-coated erythrocytes with Vero cells infected with HSV-1, EHV-1, and guinea pig herpesvirus (gpHV). Binding of EAC coated with human C3, horse C3, and guinea pig C3 to Vero cells infected with HSV-1, EHV-1 and gpHV was tested

Nevertheless there is evidence that gC of HSV-2 like type 1 gC protects virions from complement mediated lysis [28].

There are also human complement regulatory proteins, including decay accelerating factor (DAF) and membrane cofactor protein (MCP), which have low affinity for C3 deposited on foreign surfaces since they fail to form EAC rosettes, yet they are highly active in regulating the function of C3 deposited on the surface of DAF or MCP expressing cells [26]. This also suggests that rosetting may not be a useful marker to determine complement regulatory activity of the mentioned viral glycoproteins. In addition DAF seems comparable as it destabilizes the alternative pathway C3 convertase, a function which has been shown for gC of HSV-1 [11].

It is of interest that the pattern of binding noted for the interactions between gIII or its homologues and C3 correlates with the species tropism of infection. HSV-1 is a pathogen of humans but not of pigs and cows, BHV-1 causes disease

of cows but not of pigs or humans, and PRV is a pathogen of both pigs and cows but rarely infects humans.

Severe infections of humans with animal herpesviruses have been reported for B-virus, a primate herpesvirus [39] and a "natural" infection with herpes simplex virus so far only has been observed in chimpanzees [27]. This suggests that humans and these primates appear to be rather related, in terms of their herpesviruses. Preliminary data that EAC coated with macaque complement revealed good rosetting with HSV-1-infected cells (not shown) would fit with these assumptions.

Thus the species specificity of the interaction could have critical implications for choosing appropriate animal models to study HSV disease.

C3-binding proteins have been also detected on several microorganisms, including fungi [6] and protozoa [23, 31, 36]. This together with the observation that the C3-binding proteins appear to be conserved among several herpesviruses, supports the concept of an involvement of the complement-binding proteins in pathogenesis. Therefore, consideration should be given to include these C3-binding viral proteins in subunit vaccines now under development in an effort to block their participation in virus infectivity and complement regulation.

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